

DETERMINATION OF MUSHROOM MONOSACCHARIDES BY LIQUID CHROMATOGRAPHY MASS-SPECTROMETRY (LCMS) AND MACROPHAGE ACTIVATION BY POLYSACCHARIDES FROM *Polyporus umbellatus*, *Fuscoporia obliqua*, *Cordyceps militaris* AND *Pleurotus ostreatus*

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ABSTRACT

*The aims of this study are to determine the monosaccharide composition in polysaccharide and macrophage activation in cell culture by purified polysaccharide. In this study, mushroom polysaccharide was obtained from mycelia of *Polyporus umbellatus*, *Fuscoporia obliqua*, *Cordyceps militaris* and *Pleurotus ostreatus* by hot water extraction and ethanol precipitation. Polysaccharide was hydrolyzed by Trifluoacetic Acid (TFA) to get monosaccharides such as fructose, glucose, mannose and xylose. The detection of monosaccharides by Liquid Chromatography Mass-Spectrometry (LCMS) shown that fructose, glucose, mannose and xylose were only present in *P. umbellatus*. Glucose detected in *P. ostreatus* was 96.1 %, the highest compared to other species. For macrophage activation analysis, Nitric Oxide (NO) in macrophage cell culture added with polysaccharide extract from *P. umbellatus* was highest as compared to others mushroom species samples. Macrophage cell could be activated by mushroom polysaccharide and then produced NO due to antitumor activity which is harmful to cancer cells. Hence, mushroom polysaccharide has a high potential in medicinal use aspects, especially related to cancer cell study. Therefore mushroom species or strain with high in polysaccharide content is potentially beneficial for medicinal purposes. However, details studied have to be conducted for further investigation.*

Keywords: LCMS, macrophage activation analysis, monosaccharide, nitric oxide production, polysaccharide

INTRODUCTION

A polysaccharide isolated from mushroom is either water soluble or/ and insoluble glucans and heteropolysaccharides with different main and side chains. Polysaccharides are polymers of monosaccharide residues joined by glycosidic linkages; belong to a structurally diverse class of macromolecules (Lee et al., 2009). There is a great interest on these molecules as biological response modifiers. Many mushroom polysaccharides possess remarkable ability to enhance the immune system by acting as immunomodulators. Numerous studies have demonstrated the activity of polysaccharide as biological response modifiers that are able to exert beneficial effects in host by demonstrating antitumor, antibacterial, antiviral and antiparasitic activities. According to Mizuno (1995), mushroom polysaccharides have proved can enhance immune body response instead of direct cytotoxic effect on tumor cells. Polysaccharides also known for not having toxic side effects compared to existing chemical anticancer agents (Lee et al., 2009). Polysaccharides isolated from edible and medicinal mushroom have the potential to be used in medicines purpose and approaches (Rajaratnam and Vinuthna, 2014).

According to Wang et al. (2017), a wide variety of bioactive compounds from mushroom, especially medicinal mushrooms have been studied extensively. Major compounds, including polysaccharides, lectins, lactones, terpenoids, and alkaloids have been reviewed (He et al., 2017). However, among these bioactive compounds, polysaccharides with various activities are the main component for the bioactivities of some mushroom species. Polysaccharides are divided into two categories, homopolysaccharides and heteropolysaccharides based on monosaccharide composition (Wang et al., 2017).

P. umbellatus, *F. obliqua*, *C. militaris* are medicinal mushroom and *P. ostreatus* is edible mushroom. All these mushroom mainly distributed in temperate country such as in China, Japan, Europe and American. In China, dried sclerotia of *P. umbellatus* have been used as herbal medicine for more than 2000 years to cure edema and promote diuretic processes. According to Koyama et al. (2008), *F. obliqua* contains various kinds of bio-effective materials and has potential for medicinal purposes. *C. militaris* contains many kinds of active components such as cordycepin, polysaccharides, ergosterol and mannitol. Currently it is used for multiple medicinal purposes (Mizuno, 1999). Polysaccharide could be the major components in the water extract, it shown that the polysaccharide may have immune regulatory activity. The aims of this study are to detect the monosaccharide composition in some mushrooms species and macrophage activation on cell culture by purified mushroom polysaccharide.

MATERIALS AND METHODS

Mycelium Cultures

The mycelium of *P. umbellatus* ATCC 60546, *F. obliqua* NBRC 8681, *C. militaris* NBRC 9787 and NBRC 30377 and *P. ostreatus* used in this study were obtained from The Wakasa Wan Energy Research Center (WERC), Fukui, Japan.

Extraction of Polysaccharide

About 0.5 g of dried mycelium of mushroom *P. umbellatus*, *F. obliqua*, *C. militaris* and *P. ostreatus* were put into a falcon tube separately. Eight milliliter of distill water was added into each falcon tube and then incubated at 90°C for 4 hours. The mycelium was filtered and the solution was transferred into a new falcon tube. Distill water was added to adjust volume of solution into 7.5 ml. Four volumes of ethanol were added then incubated at 4°C for overnight. The falcon tube was centrifuged at 110,000 rpm for 15 min. The supernatant was discarded and then dried using centrifuge vaporization for 4 hours. Distill water was added into a falcon tube about 1% of the dried weight of the polysaccharide.

Hydrolyze of Polysaccharide and Detection by LCMS

The polysaccharide was hydrolyzed to get monosaccharides by adding 1 ml of distill water with 100 µl of Trifluoroacetic acid (TFA) into 100 µl of polysaccharide. The mixture was incubated in water bath at temperature 120°C for 1 hour. After 1 hour, distilled water was added to prepare 1000 times dilution. The composition of monosaccharide was detected by LCMS using column Suger-D 4.6DX250 mm at temperature 30°C with flow rate at 0.9 ml/min. The eluent composition consisted of 78% acetonitrile and 22% ammonium acetate.

Macrophage Cell Culture

The mouse macrophage cell RAW246.7 used in this study was purchased from American Type Culture Collection (ATCC). The macrophage cell RAW246.7 was maintained in culture medium consists of Dulbecco's Modified Eagle's Medium (DMEM), serum and 2 M HEPES then incubate in 5% CO₂ incubator.

Cell Viability by Polysaccharide

About 100 mg/L of polysaccharide extract of mushroom *P. umbellatus*, *F. obliqua*, *C. militaris* and *P. ostreatus* were added into different petri dish containing macrophage cell with DMEM without color. Petri dish contained macrophage cell and polysaccharide was incubated in 5% CO₂ incubator for overnight.

Determination of NO Production

Supernatant from incubated macrophage cell and polysaccharide was collected and measured by spectrophotometer at 540 nm wavelength by adding 500 µl of Griess reagent. The control sample of macrophage cell was incubated without polysaccharide.

RESULTS AND DISCUSSION

Composition of Monosaccharide

The retention times of monosaccharide standard for glucose, fructose, mannose and xylose from LCMS data are shown in Figure 1. The monosaccharides were detected in polysaccharide from *P. umbellatus*, *F. obliqua*, *C. militaris* and *P. ostreatus* as shown in Table 1. The polysaccharide from *P. umbellatus* consists of 26.2% fructose, 71.6% glucose, 1.0% mannose and 1.2% xylose. Polysaccharide from *F. obliqua* and *C. militaris* consist only fructose, glucose and mannose. According to Wang et al. 2017, monosaccharides composition for *C. militaris* usually consisted of glucose, mannose and galactose. However, the monosaccharide standard for galactose was not used in this study. Meanwhile *P. ostreatus* only consist glucose and mannose with ratio 96.1%: 3.9%.

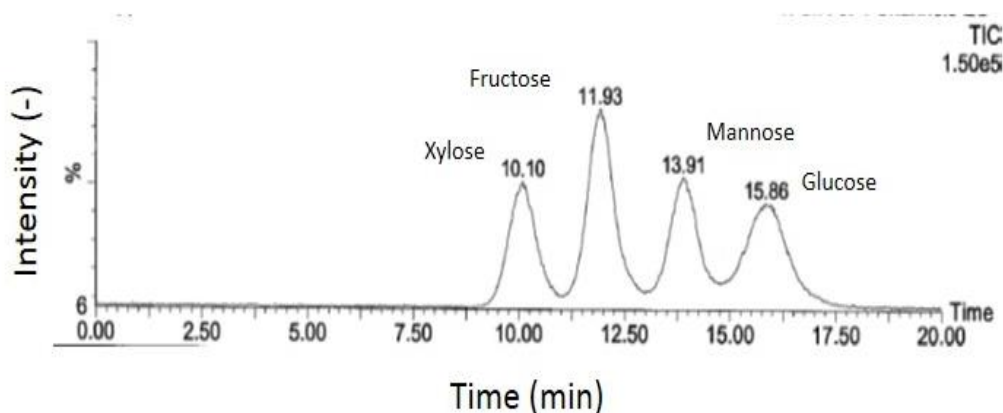


Figure 1: Retention time of monosaccharides standard mixture

Table 1: Monosaccharide composition on mushroom polysaccharide

Mushroom Species	Composition Ratio of Monosaccharide (%)			
	Fructose	Glucose	Mannose	Xylose
<i>P. umbellatus</i>	26.2	71.6	1.0	1.2
<i>F. obliqua</i>	13.9	84.7	1.5	0
<i>C. militaris</i> NBRC 9787	2.7	83.6	13.7	0
<i>C. militaris</i> NBRC 30377	6.7	86.1	7.2	0
<i>P. ostreatus</i>	0	96.1	3.9	0

From these results, the use of Suger-D 4.6D x 250 mm column with eluent composition of acetonitrile 78% and ammonium acetone 22% have given a good resolution with a small volume of sample (Figure 2).

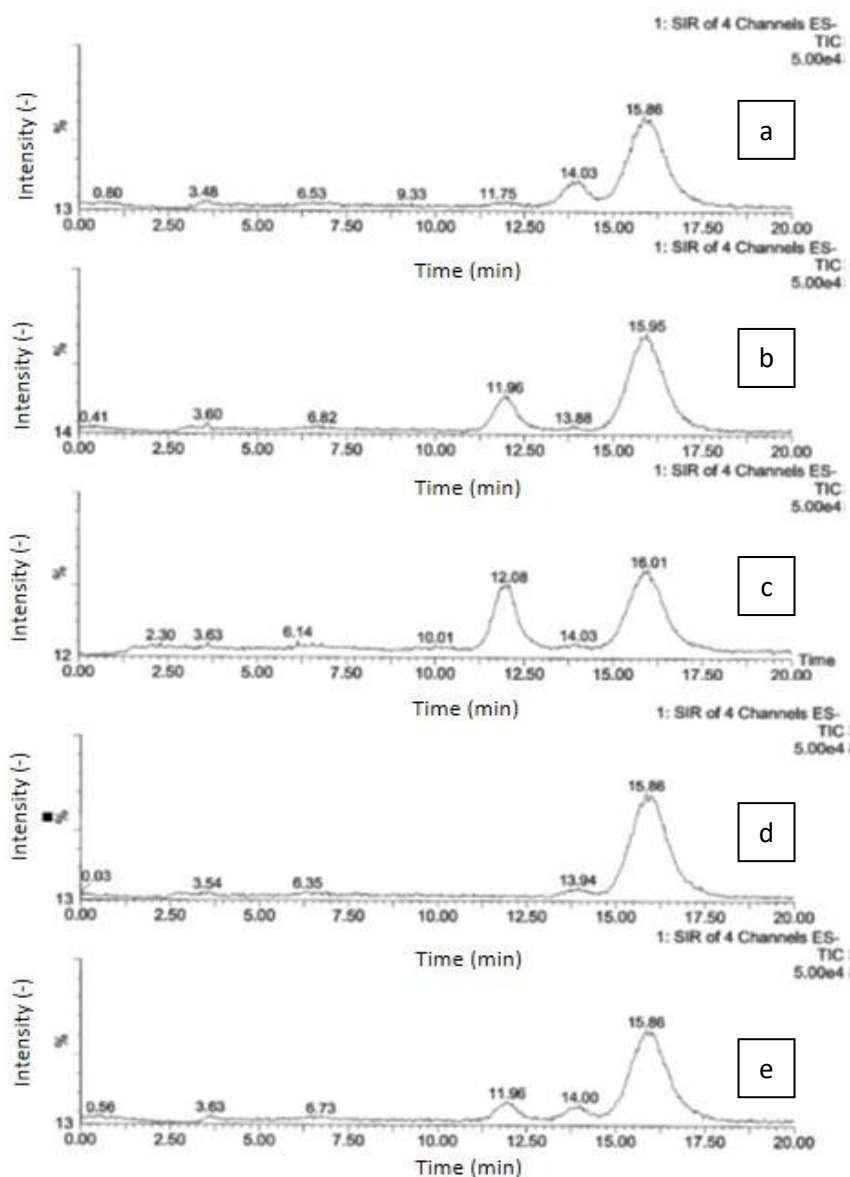


Figure 2: Chromatorgrams of monosaccharides separation for a) *P. umbellatus*, b) *F. obliqua*, c) *C. militaris* NBRC 9787, d) *C. militaris* NBRC 30377 and e) *P. ostreatus*

Macrophage Activation by Polysaccharide

The results of NO production in macrophage cell culture with polysaccharide from *P. umbellatus*, *F. obliqua*, *C. militaris* NBRC 9787, *C. militaris* NBRC 30377 and *P. ostreatus* are shown in Figure 3. *P. umbellatus* showed highest NO production compared with other species. The NO production for *C. militaris* strain NBRC 30377 is higher with 31.70 μM as compared to NBRC 9787 with 21.87 μM .

According to Lee et al., 2009, activated macrophages play an important role in innate and adaptive immune responses by producing cytokines such as IL-1 β and TNF- α , NO, and other inflammatory mediators. Therefore, determination of NO production can be used as an indicator in the study of immune responses in the cell. The production of these mediators is an important part of the immune response to many inflammatory stimuli (Porcheray et al., 2015).

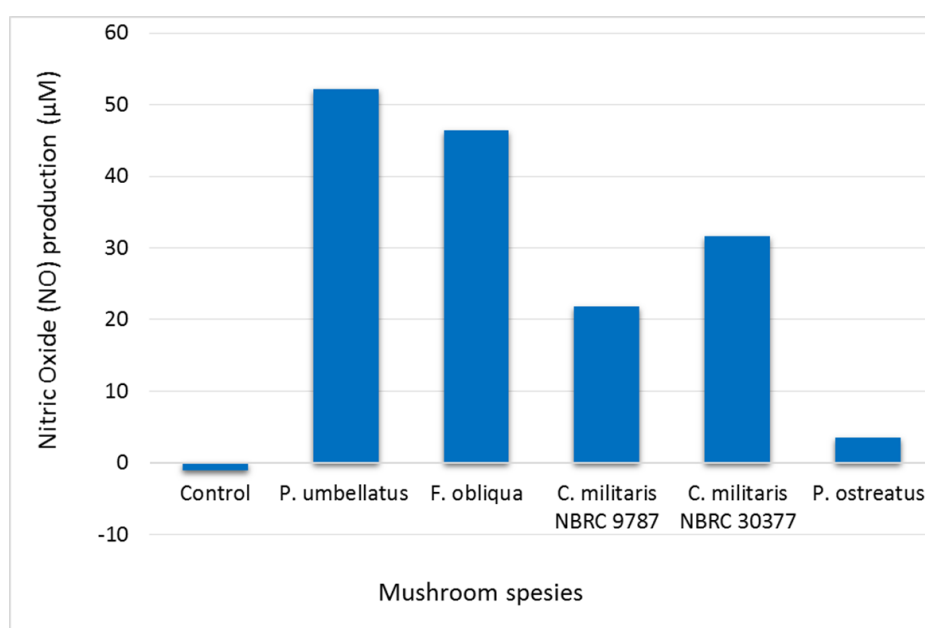


Figure 3: NO production in macrophage cell culture with polysaccharides from *P. umbellatus*, *F. obliqua*, *C. militaris* NBRC 9787, *C. militaris* NBRC 30377 and *P. ostreatus*

CONCLUSIONS

Monosaccharide composition or combination plays an important role in biological activity. However, the bioactivity mechanisms, biosynthetic pathways, and productivities of the polysaccharides are highly variable and confusing to researchers (Wang et al., 2017). The monosaccharides of fructose, glucose and mannose were detected in *P. umbellatus*, *F. obliqua*, *C. militaris* and *P. ostreatus*. However, xylose was only detected in *P. umbellatus*. The NO production in macrophage cell culture with polysaccharide *P. umbellatus* was shown the highest compared with other species. The finding from this preliminary study is a preamble for further works on mushroom polysaccharides for medicinal approaches. Therefore, more detailed studies along this line have to be conducted for further understanding and knowledge.

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